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Review

On-line sample preconcentration techniques in micellar electrokinetic chromatography

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

This review provides an overview as well as a practical understanding of on-line sample concentration techniques in micellar electrokinetic chromatography (MEKC). MEKC as well as other capillary electrophoretic modes suffer from low concentration sensitivity due to minute sample volume and limited optical pathlength for on-capillary photometric detection. Two on-line sample preconcentration techniques, sample stacking and sweeping are known to be effective techniques for enhancement of the concentration sensitivity in MEKC. Sample stacking occurs as ions cross a boundary that separates regions of the high electric field sample zone and the low electric field background solution zone. The difference in migration velocity of pseudostationary phases within the two zones is the key to achieving the focusing effect. Sweeping is defined as the picking and accumulating of analytes by the pseudostationary phase that penetrates the sample zone devoid of pseudostationary phase. In this review, several examples of the sample stacking and sweeping under different experimental conditions are given, besides many references to applications.

Keywords: Reviews; Micellar electrokinetic chromatography; Sample stacking; Sweeping

1. Introduction

In recent years, capillary electrophoresis (CE) has been developed as a separation analysis method suitable for routine applications. Its popularity may be attributed to its extremely high efficiency, short analysis time, and wide application range. The basic modes of CE that have been matured and are presently being exploited include capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary isotachophoresis (ITP), capillary isoelectric focusing, capillary gel electrophoresis, and capillary electrochromatography. In CZE, only ionic or charged analytes can be analyzed in principle, since its separation mechanism is based on the

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difference in electrophoretic mobilities of analytes. MEKC [1] has become popular as a powerful technique for improving separation efficiency not only of neutral analytes but charged ones by using a CE instrument without any alteration. In MEKC, an ionic surfactant is used as a pseudostationary phase (PS) that corresponds to the stationary phase in conventional chromatography and the surrounding aqueous phase to the mobile phase. The separation principle of analytes is based on their differential partitioning between the aqueous phase and the micelle phase.

The most widely used detector in CE is the UV phorometric detector, because many solutes have UV absorption and the UV detector is easily set up and is cost-efficient. One of the drawbacks of UV-detection MEKC, as in the other modes of CE, is the poor concentration sensitivity resulting from minute injection volumes needed to maintain high efficiency and a short optical pathlength equal to the capillary diameter. This hinders the applicability of MEKC for the analysis of dilute analyte mixtures. Thus, method development is indispensable for reducing limits of detection (LODs) or increasing concentration sensitivity. Currently, solving the problems associated with low concentration sensitivity has been the emphasis of various reports. These investigations involve the installation of capillaries equipped with extended detection pathlength (e.g. Z-shaped, multi-reflection, and bubble cell), the use of highly sensitive detection methods (e.g. laser-induced fluorescence, electrochemical, and amperometric detection), and sample preparation methods (e.g., liquid–liquid and/or solid-phase extraction). However, all these methods require rather expensive and somewhat complex hardware or time consuming procedures. Of considerable interest to improve detection sensitivity in MEKC is the development of on-line sample concentration techniques, such as sample stacking [2–21] and sweeping [22–45] that are performed individually or in combination.

In this article, two on-line sample concentration techniques, sample stacking and sweeping, in MEKC using various surfactants are reviewed. Their mechanism and applications under different experimental conditions are also discussed.

2. Micelles as pseudostationary phase for on-line preconcentration

The separation of electrically neutral solutes by MEKC is accomplished by the surfactants in the background solution (BGS). Surfactants are molecules, which show both hydrophobic and hydrophilic character. They have polar head groups that can be anionic, cationic, nonionic, or zwitterionic; and nonpolar, hydrocarbon tails. When a surfactant concentration in separation solution is higher than the critical micelle concentration (CMC), individual surfactant molecules aggregate to form micelles. Micelles have a dynamic structure that is the result of the rapid equilibrium between aggregated and monomeric forms. It is differences in interaction between the micelle and the neutral solute that cause the separation.

Many neutral and charged analytes have been concentrated based on the diverse concentration techniques by MEKC. Various kinds of surfactants as PSs for the on-line sample concentration in MEKC are summarized in Table 1. An anionic surfactant, sodium dodecyl sulfate (SDS) is the most widely used. Cationic surfactants such as cetyltrimethylammonium chloride (CTAC) and tetradecyltrimethylammonium bromide (TTAB), and nonionic surfactants, such as polyoxyethylene (23) dodecyl ether (Brij 35) and polyoxyethylene (20) sorbitan monolaurate (Tween 20) have also been effectively used as PSs for on-line concentration techniques. Moreover, macromolecular surfactants, butyl acrylate–butyl methacrylate–methacrylic acid copolymers (BMMA) and sodium 10-undecylenate (SUA) oligomer, and bile salt (e.g. sodium cholate, SC) have been introduced as PSs. Although microemulsion is different but similar and cyclodextrin (CD) derivative do not form micelles, they have also been employed as PSs for concentration techniques in MEKC. Explanation and examples of each PS for on-line sample concentration are discussed later.

Table 1
Conditions of sample stacking modes in MEKC using SDS micelle

Modes	Sample matrix	Water plug[a]	pH (BGS)	EOF[b]	Polarity
Hydrodynamic injection					
NSM	Low conductivity (non-micellar)	×	Basic or neutral	Cathodic (strong)	+
REPSM	Low conductivity (non-micellar)	×	Basic or neutral	Cathodic (strong)	−→+[c]
SRMM	Low conductivity (non-micellar)	×	Acidic	Cathodic (weak)	−
SRW	Low conductivity (micellar)	○	Acidic	Cathodic (weak)	−
Electrokinetic injection					
FESI	Low conductivity (micellar)	○	Basic or neutral	Cathodic (strong)	−→+[d]
FESI-RMM	Low conductivity (micellar)	○	Acidic	Cathodic (weak)	−

[a] A water plug is introduced into the capillary at the inlet end after conditioning the capillary with BGS.
[b] EOF is suppressed under the acidic conditions.
[c] Polarity is to be switched when current reached close to 97% of the original value.
[d] Electrokinetic injection with negative polarity, then separation voltage is applied at positive polarity.

3. Sample stacking

3.1. Fundamental

Sample stacking technique was first introduced for ionic analytes in CZE [46,47]. Thereafter, a variety of on-line sample concentration techniques have been reported in CZE. They are field-amplified sample stacking [46,48], large-volume sample stacking [49,50], pH-mediated stacking [51,52], ITP [53,54], and dynamic pH-junction [55,56]. Recently, Beckers et al. [57] and Quirino and Terabe [58] have published comprehensive reviews.

The basic model of sample stacking is illustrated in Fig. 1. In CZE (Fig. 1A), the sample is dissolved in a solution that has a lower conductivity than that of the BGS and injected as a long plug than that in normal injection. Sample stacking occurs as ions cross a boundary that separates regions of the high electric field sample zone and the low electric field BGS zone. The sample and BGS zones are the low- and high-conductivity zones, respectively. When separation voltage is applied, the electric field strength experienced in the sample zone is higher than in the rest of the capillary. The sample ions in the sample zone will move quickly, and then slow down when they reach the BGS zone because they experience lower electric field strength. Consequently, the ions are focused at the boundary of the two zones. It should be noted that electroosmotic flow (EOF) is assumed to be zero in Fig. 1A. However, this technique cannot be applied to neutral analytes because neutral analytes have no electrophoretic mobility. In MEKC (Fig. 1B), to give effective electrophoretic mobilities to neutral analytes, charged PSs (e.g. SDS) are employed. The sample solution is prepared by dissolving the neutral analytes in a low conductivity solution and injected into the capillary, which has been previously conditioned with anionic SDS micellar BGS at neutral pH. The neutral analytes in sample solution can be quickly carried to the boundary between the BGS and sample solution by the fast migrating anionic micelle entering into the sample solution from the cathodic end. Since the electric field strength in the BGS zone is low, the velocity of the micelles is retarded, and the analytes are focused at the boundary between BGS and the anodic end of the sample solution zone. Note that the neutral analytes are brought to the detector by the EOF since the magnitude of the EOF is greater than the electrophoretic velocity of the micelle.

The degree of sample stacking and enhancement of concentration is proportional to the ratio of resistivities of the sample solution and BGS. In a previous paper [47], the field enhancement factor, γ, is given as

$$\gamma = C_{BGS}/C_S$$

where C_{BGS} is the analyte concentration in the BGS and C_S is that in the sample solution.

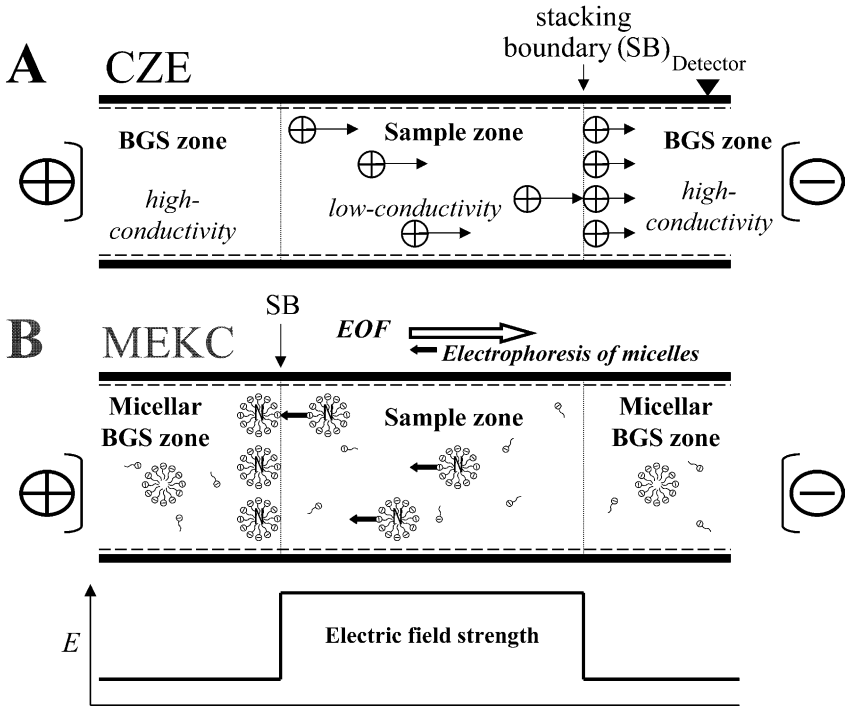

Fig. 1. Schematic diagram of the principle of sample stacking in CZE (A) and MEKC (B). See text.

Theoretically, the degree of stacking is simply proportional to γ. The larger the difference in concentrations, the narrower peaks, and the more efficient stackings are. If the samples are dissolved in water, γ can be several hundreds. However, a partial laminar flow is produced inside the capillary due to the difference in electric field strengths between the sample and BGS zones, which cause different electroosmotic velocity in each zone. This laminar flow will broaden the narrow zone generated by sample stacking process. The larger the difference in concentrations, the greater the laminar flow will be. Stacking and zone broadening oppose each other, causing an optimal length of sample plug that can be introduced into the column and still achieve high resolution [48].

3.2. Stacking modes

Conditions of different stacking modes in MEKC using SDS micelle are summarized in Table 1.

3.2.1. Normal stacking mode (NSM)

NSM [8] is the simplest sample stacking technique and the principle of NSM with an anionic micelle is illustrated in Fig. 1B. Analytes prepared in a low-conductivity matrix are injected into the capillary, after conditioning the capillary with micellar BGS. When a positive separation voltage is applied, micelles from the BGS migrate towards the anode, enter the sample zone, enhance their migration velocity in this zone and focused as described above. After stacking, analyte zones separate by virtue of MEKC. The principle of NSM with a cationic surfactant is the same as that with an anionic surfactant except for the electrode polarity [36].

3.2.2. Reversed electrode polarity stacking mode (REPSM)

In REPSM [9], the capillary is conditioned with micellar BGS and the analytes prepared in low-conductivity matrix are injected for a much longer time compared to NSM. Separation voltage is then applied at negative polarity (positive at outlet).

Micelles from the cathodic vial will carry and stack the neutral analytes at stacking boundary (SB), which is the interface between the sample zone and BGS, and the sample matrix is pumped out from the capillary by EOF. The current shift should be monitored carefully and the current is first gradually increased with the removal of the sample matrix. When the measured electric current reaches 97–99% of the predetermined value, the polarity is switched to positive. The polarity-switching procedure allows a large volume of sample matrix after stacking to be removed, however, this method involves a delicate operation to monitor the current carefully and leads to poor reproducibility if the current is not monitored well. The dispersive effect can be minimized in REPSM by pumping out the sample matrix by applying negative voltage. Here, negative polarity is needed to focus the analytes and positive polarity is needed to separate the analytes.

3.2.3. Stacking with reverse migrating micelles (SRMM)

SRMM technique [10] employs an acidic micellar BGS to reduce the EOF. Under these conditions, the electrophoretic velocity of SDS micelles is higher than that of the EOF [59]. Samples prepared in purified water or in low conductivity matrix are injected for a much longer time compared to the normal injection, after conditioning the capillary with micellar BGS. Sample solutions are introduced at the cathodic end of the capillary and then separation voltage is applied with negative polarity at the injection end. Since the negative polarity is applied at the inlet, the sample matrix was slowly pushed out of the capillary by the weak EOF. Unlike REPSM, only negative polarity is needed to focus and separate the analytes without the polarity-switching step in SRMM. The technique is very effective also in CZE [60].

3.2.4. Stacking using reverse migrating micelles and a water plug (SRW)

In SRW [11], samples are also prepared in a matrix having a conductivity lower than the BGS, however, the surfactant is added at a concentration slightly higher than its CMC to increase solubility of analytes. Unlike NSM, REPSM, and SRMM, a water plug is introduced into the capillary at the inlet end after conditioning the capillary with BGS at acidic pH followed by the injection of a long sample plug. The water plug provides the low-conductivity zone, which has higher electric field strength compared to the sample solution. The stacking mechanism is primarily based on the abrupt change in effective electrophoretic velocities at the SB. Separation voltage is then applied at negative polarity with BGS both in the cathodic and anodic vials. Focusing of analytes zones, removal of the sample matrix and water plug, and separation of focused analyte zones occur subsequently. SRW complements the SRMM for the concentration of sparingly water soluble analytes in terms of sample preparation.

3.2.5. Field-enhanced sample injection (FESI)

In FESI [12], a water plug is introduced at the inlet end of capillary filled with BGS at neutral pH. The water plug provides an enhanced electric field zone for effective stacking. The sample is prepared in a low-conductivity micellar matrix at neutral pH, and then electrokinetically injected at negative polarity. During injection, the analytes solubilized in the micelles enter the capillary together with micelles from the sample vial owing to the enhanced field in the water plug that generates electrophoretic velocities greater than the EOF. Once the current has reached 97–99% of its original value, that is, when the water plug has been considerably removed from the capillary, the voltage is shut off and the sample vial is replaced by BGS vial. Separation voltage is applied at positive polarity to perform separation and detection.

3.2.6. Field-enhanced sample injection with reverse migrating micelles (FESI-RMM)

In FESI-RMM [13], to effect an enhanced field at the injection end of capillary, a water plug is injected into the capillary end using pressure. The BGS has an acidic pH. The sample is also prepared in low-conductivity micellar matrix at acidic pH. Injection voltage is applied at negative polarity with sample solution. The sample vial is replaced

by the BGS, when 70–90% of the original current (depending on the kind of analyte and analytical parameters) is reached. Then separation voltage is applied at negative polarity until all peaks are detected. In FESI-RMM, similar to SRMM, no polarity switching is necessary and only the sample vial is replaced by a BGS vial after the predetermined optimum injection time is reached.

3.2.7. Other techniques

ITP-MEKC technique has been reported by Enlund and Westerlund [6]. It is a moving boundary technique in which the analyte is concentrated between two zones consisting of leading and terminating buffers. Selection of leading and terminating buffers has an important part in separation and concentration. Zhang and Thormann [14] reported preliminary results of head-column sample stacking in MEKC. In this technique, a plug of high conductivity solution devoid of micelle is introduced prior to sample injection. The micelle-free plug prevents SDS micelles from penetrating into the sample zone during electrokinetic injection. Further investigations on the stacking mechanism with charged micelles are required.

3.3. Applications

Applications of sample stacking and sweeping in MEKC are summarized in Table 2.

3.3.1. Anionic micelles

The anionic surfactant SDS is the most widely used for on-line sample concentration techniques. Its popularity can be attributed to its low CMC, high aqueous solubility, low Krafft point, ready availability and low cost of pure product. For concentration techniques, moreover, the EOF is suppressed significantly below pH 5.5 [59].

Three environmentally important phenylurea herbicides were separated and concentrated by SRMM MEKC using anionic SDS micelle [18]. Moreover, coupling of solid-phase extraction to SRMM provided further sensitivity enhancements. About 1 ppb of each herbicide spiked in tap or pond water was detected. Fig. 2 shows the SRMM-MEKC analysis of phenylurea herbicides spiked in tap water after solid-phase extraction.

Separation and concentration of several dioxins and related compounds by SRW MEKC are shown in Fig. 3 [16]. CD modified MEKC was employed because of the high hydrophobicities of polychlorinated dibenzo-*p*-dioxins. By applying the SRW technique, the LOD was successfully decreased to around 0.1 ppm, translating to about 200-fold enhancement detection sensitivity.

3.3.2. Cationic micelles

Cationic surfactants such as CTAC and TTAB were also employed for concentration techniques. Most cationic surfactants have an alkyltrimethylammonium group and their counter-ions are halides. The addition of cationic surfactants to the BGS caused the reversal of EOF owing to positively charged capillary wall by the adsorption of cationic surfactants [61,62]. As a result of the reversed EOF, polarity of the electrodes must have to be reversed in order to detect the analytes. Fig. 4 shows an example of NSM using a cationic surfactant and compares the peak heights and shapes between different sample matrices [28]. Fig. 4A shows an electropherogram of conventional MEKC. As shown in Fig. 4B, where the analytes were dissolved in the micellar BGS and then injected for 30 s, broader peaks were observed without significant improvement of peak height. This was due to the similar conductivities between the sample matrix and BGS. Because of the presence of the micelle in the sample matrix, thus no stacking effect occurred. On the other hand, when the analytes were dissolved in low-conductivity matrix, more than 10-fold enhancements of peak heights were obtained by sample stacking (Fig. 4C). In NSM, the injected length of the sample zone was limited by the dispersive effect brought about by the local electroosmotic velocity mismatch between the low- and high-conductivity zones [8,63].

3.3.3. Macromolecular micelles

Macromolecular surfactants or high molecular mass surfactants (HMMS), BMMA and SUA oligomer have been introduced as PSs for concentration techniques in NSM, REPSM, and FESI

Table 2
Applications of sample stacking and sweeping in MEKC

Mode	PS	Analyte	LOD	SEF	Ref.
Sample stacking					
NSM	SDS, BBMA, SUA	Resorcinol, 1-naphtol, 2-naphtol	$5.08–7.03 \times 10^{-7}$M	10–12	[8]
	SDS	Triazines	NS	NS	[17]
	SDS	Iso-α-acids	0.09–0.38 ppm	NS	[3]
	SDS	Tetrachlorodibenzo-*p*-dioxins	NS	NS	[4]
	CTAC	Nitrobenzene, resorcinol, 2-naphtol	$2.77–7.15 \times 10^{-7}$M	13–15	[28]
	Brij 35	Phenols	NS	40	[33]
REPSM	SDS	Tetrachlorodibenzo-*p*-dioxins	NS	75–85	[4]
	SDS, BBMA, Brij 35, SUA	Resorcinol, 1-naphtol, 2-naphtol, 1,6-dihydroxynaphthalene	NS	28–45	[9]
	SDS	Triazines	3.3–8.5 ppb	NS	[17]
	SDS	Plant hormones	0.31–8.2 ppb	NS	[21]
SRMM	SDS	Phenols	NS	18–131	[10]
		Steroids	13.8–19.3 ppb	NS	
	CTAC	Steroids	NS	56–68	[27]
	Heptakis(6-sulfato)-β-CD	Phenoxy acid herbicides	NS	90–158	[24]
SRW	SDS	PAHs	NS	28–43	[11]
		Steroids	NS	72–102	
	SDS	Dioxin-related compounds	0.1 ppm	200	[16]
FESI	BBMA	Estrogens	NS	60–106	[12]
FESI-RMM	SDS	Alkyl phenyl ketones	NS	2–18	[13]
		Phenols	NS	3–43	
		Steroids	8–12 ppb	41–78	
ITP-MEKC	Tween 20	Methylamitriptyline, amitriptyline, nortriptyline	NS	NS	[6]
Head-column	SDS	Metabolites of codein and dihydrocodein	NS	NS	[14]
Sweeping					
	SDS	Alkyl phenyl ketones	NS	88–836	[22]
		Naphthalene derivatives	NS	92–259	
		Dialkyl phthalates	NS	272–691	
		Steroids	1.7–9.6 ppb	1541–2564	
		Reserpine, nicardipine, quinine, trimipramine	NS	3312–5044	
	SDS	Proguanil, 4-chlorophenylbiguanide, cycloguanil	10–20 ppb	NS	[29]
	SDS	Estrogens	53–100 nM	NS	[32]
	SDS	Doxorubicin, daunorubicin	1 nM	NS	[34]
	TTAB	*o*-, *m*-, *p*-Nitroanilines	NS	20	[28]
		Aromatic carboxylic acids	NS	1000	
		NSAs	0.47–0.96 ppb	670–760	
	TTAB	Steroids	9–20 ppb	270–370	[27]
	CTAC	2-Naphthoic acid, salicylic acid	0.4–3.1 ppb	590–600	
	TTAB	Triazines	9–15 ppb	30–110	[30]
	Brij 35	4-Chlorophenol, 4-ethylphenol, 3-methylphenol	19–28 ppb	54–100	[33]
	Microemulsion	Steroids	NS	138–278	[24]
	poly SUS	Heptanophenon, quinine	NS	50–230	[36]
	pSAm-28	Quinine	42 ppb	580	
	pSAm-24	Quinine	5–3 ppb	5800–10 000	

Table 2 (*Continued*)

Mode	PS	Analyte	LOD	SEF	Ref.
	SC	Corticosteroids	50 ppb	NS	[37]
	SC	Estrogens	118 ppb	NS	[38]
Selective exhaustive injection-sweeping					
CSEI-sweep	SDS	Laudanosine, 1-naphthylamine	4.1–8.0 ppt	550 000–900 000	[40]
	SDS	Aromatic amines	0.1 ppt	12 000–146 000	[41]
	SDS	Paraquat, diquat, difenzoquat	0.075–1.0 ppb	3000–51 000	[42]
ASEI-sweep	CTAC	Aromatic carboxylic acids	NS	2400–2700	[31]
		Dansyl amino acids	0.8–1.2 ppb	1150–1430	
		Naphthalenedisulfonic acids	60–80 ppt	5800–5900	
Stacking-ESI-MS					
	SDS	*N*-Methylcarbamate pesticides	0.04–2.0 ppm	NS	[68]
Sweeping-APCI-MS					
	SDS	Amines	0.6 ppm	100–300	[44]
		Dialkyl phthalates	0.4 ppm	70–600	
Microchip-stacking					
	SC	BODIPY	NS	20	[78]
Microchip-sweeping					
	SDS	Rhodamine B, sulforhodamine B, Cresyl fast violet	NS	90–1500	[45]

Abbreviations: LOD, limit of detection; SEF, sensitivity enhancement factor; NS, not stated; BBMA, butyl acrylate–butyl methacrylate–methacrylic acid copolymers; SUA, sodium 10-undecylenate oligomer; Brij 35, polyoxyethylene (23) lauryl ether (Brij 35); PAH, polycyclic aromatic hydrocarbon; Tween 20, polyoxyethylene (20) sorbitan monolaurate; poly SUS, poly(sodium 10-undecenyl sulfate); pSAm-24 (or 28), poly(2-acrylamido 2-methyl propane sulfonic acid-co-stearylacrylamide) (where 24 or 28 indicates the mole percentage of the hydrophobic monomer in the polymer); BODIPY, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propanol.

MEKC. Since an HMMS forms a molecular micelle, which consists of one molecule, and the CMC value is essentially zero, one can expect a higher reproducibility in an HMMS–MEKC system compared to a low molecular mass surfactant (LMMS)–MEKC system.

In stacking modes, the surfactant concentration in low conductivity zone is low due to enhanced velocity of the micelles and hence the micellar concentration must be low because CMC is constant. Since CMC of HMMS is essentially zero, the micellar concentration must be relatively high in the sample zone even in stacking mode. Thus, we expect high concentration efficiently when HMMS is employed in stacking mode.

In REPSM MEKC, the effect of the nature of three PSs (SDS, SUA, and BBMA) to stacking was well demonstrated [9]. Detection limits calculated using BBMA and SUA were much lower than those calculated using a SDS. In FESI, moreover, only HMMS were found to be of value [12]. To date, the problem with macromolecular surfactants is their low chemical purity.

3.3.4. Cyclodextrin (CD)

CD has been widely used not only in liquid chromatography but also in CE as a mobile phase modifier. The use of CD is particularly effective for the separation of aromatic isomers and enantiomers, which have the chiral center close to the aromatic ring. Here, the abbreviation CDEKC is used for electrokinetic chromatography (EKC) using CD as a PS, and in order to describe techniques in a general manner, the term PS is

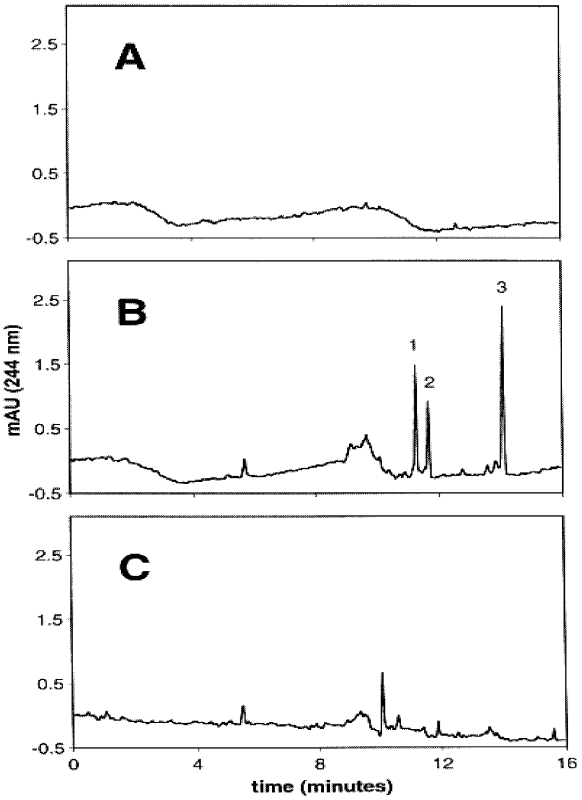

Fig. 2. SRMM-MEKC analysis of phenylurea herbicides spiked in tap water after solid-phase extraction (SPE). BGS, 50 mM SDS–50 mM phosphoric acid–15 mM β-cyclodextrin; Herbicides in sample, isoproturon (1), diuron (2), monuron (3); concentration of herbicides in tap water, 1 ppb each (A, B), blank (C); injection, 1.2 s (A), 100 s (B, C); capillary diameter, 50 μm I.D.; separation voltage, −20 kV; SPE eluting solvent, acetone.

used instead of the term micelle used above. Fig. 5 shows the electropherograms obtained for separation and concentration of phenoxy acid herbicides and their enantiomers using heptakis(6-sulfato)-β-CD as PS [24]. Here, three sample stacking techniques [stacking with reverse migrating pseudostationary phase (SRMP), field-enhanced sample injection with reverse migrating pseudostationary phase (FESI-RMP) and stacking using reverse migrating pseudostationary phase and a water plug (SRW)] and sweeping were evaluated. More than 100-fold sensitivity enhancements were obtained by SRMP CDEKC. These results show the utility of CD as PS for on-line sample concentration in EKC.

4. Sweeping

4.1. Fundamental

Sweeping technique was first described in EKC using a charged PS (i.e. micelle). Sweeping is defined as a phenomenon where analytes are picked up and concentrated by the PS that penetrates the sample zone devoid of PS. It is independent of the EOF and effective for both charged and uncharged solutes.

The principle of sweeping under the acidic condition is schematically shown in Fig. 6. In step A, test analytes prepared in a matrix, with conductivity similar to that of BGS but devoid of PS, are pressure injected into the capillary at the cathodic end. In step B, once the separation voltage is applied at the negative polarity with the BGS in the inlet vial, anionic PS will enter the capillary and sweep the analytes. In step C, the analytes are completely swept by the PS and followed by MEKC separation in the reverse migration mode.

In sweeping, the length of the resulting zone after sweeping (l_{sweep}) is given by [22]

$$l_{sweep} = l_{inj}\frac{1}{1+k}$$

where l_{inj} is the length of the injected sample zone and k is the retention factor of analyte. The k values in the sample zone when filled with PS are assumed equal to those in the separation zone. Sweeping is then basically dependent on the retention factor and the length of the initial zone. Thus, it is suitable for strongly retained analytes.

Recently Palmer et al. reported *micellar stacking* with a high-salt concentration sample matrix affording high concentration efficiency [37–39], although we think the technique is equal to sweeping in total [26,43]. The reason why high-salt concentration sample matrices can produce a high concentration efficiency may be explained by the fact that retention factors are significantly

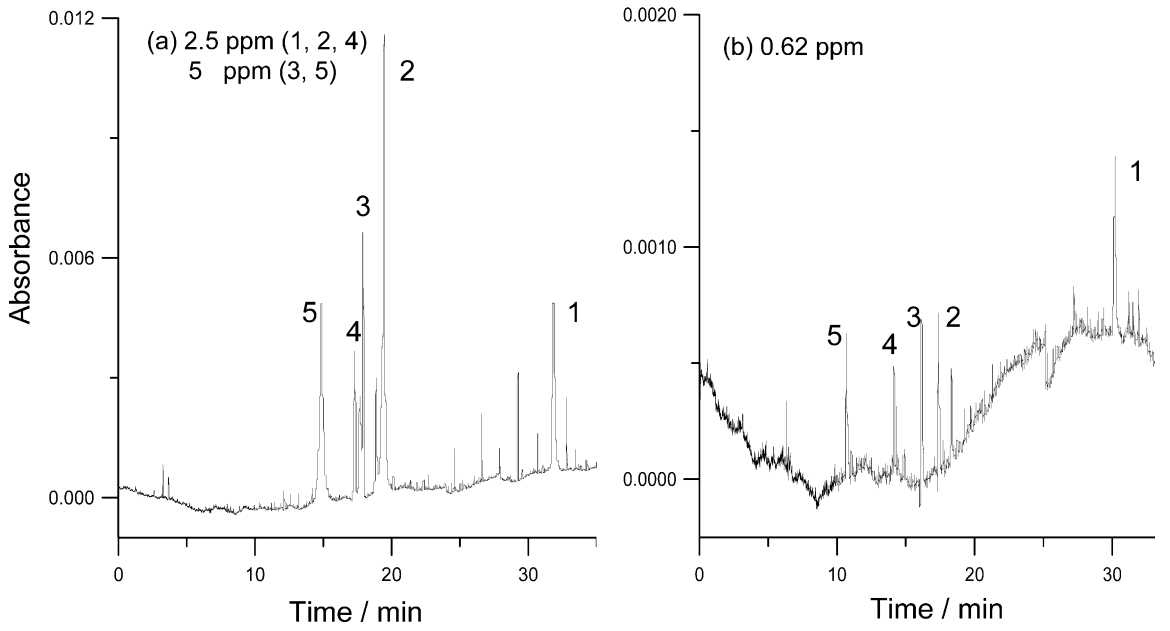

Fig. 3. Separations of the test solutes by SRW–CD-MEKC. Separation solution, 100 mM SDS–40 mM β-CD in 50 mM phosphate buffer (pH 2.5) containing 5 M urea; injections, (a) water plug, 300 s and sample, 300 s, (b) water plug, 500 s and sample, 200 s; peaks, (1) 2,3-dichlorodibenzo-*p*-dioxin, (2) dibenzofuran, (3) 2,3,7-trichlorodibenzo-*p*-dioxin, (4) dibenzo-*p*-dioxin, (5) 2,7-dichlorodibenzo-*p*-dioxin; detection wavelength, 225 nm; solute concentrations, see text.

increased in high-salt concentration matrices [43]. Sweeping mechanism works under any salt concentrations of sample matrices, provided that the sample matrix is devoid of the PS.

4.2. Applications

4.2.1. Anionic micelle

Like in sample stacking techniques, SDS micelle is also widely used for sweeping of neutral and cationic analytes. In a previous paper [22], because of electrostatic interactions between cationic analytes and anionic SDS micelles, about 5000-fold improvement in detector response of some weakly basic and hydrophobic drugs has been reported. Proguanil and its metabolites in plasma and urine have been successfully separated and concentrated by sweeping using SDS micelle [29]. Harino et al. [32] reported a method combining solid-phase extraction with sweeping for the analysis of three estrogens (estrone, β-estradiol and ethynylestradiol) at low levels in water. LOD of each analyte could be lowered by factor of about 1000- and 300 000-fold by sweeping and solid-phase extraction followed by sweeping, respectively. Fig. 7 shows an example electropherogram of sweeping MEKC using SDS micelle [22].

4.2.2. Cationic micelles

From the viewpoint of the sweeping theory, the use of the cationic micelles is straightforward for concentration of anionic analytes [27,28]. Negatively charged analytes, naphthalenesulfonic acids (NSAs), were subjected to sweeping MEKC using cationic CTAC micelle (Fig. 8) [28]. Highly sensitive analysis of NSAs in water sample is of great concern in the field of environmental analysis. Fig. 8A shows the electropherogram of normal injection (0.58 mm injected). The electropherogram obtained after the sweeping (45.6 cm injected) is depicted in Fig. 8B. When more of the sample solution was injected, peak heights leveled off and peaks showed incomplete separation. This is considered to be a result of the sample zone passing the detector before the complete concentration. As a result, the obtained LODs of the test

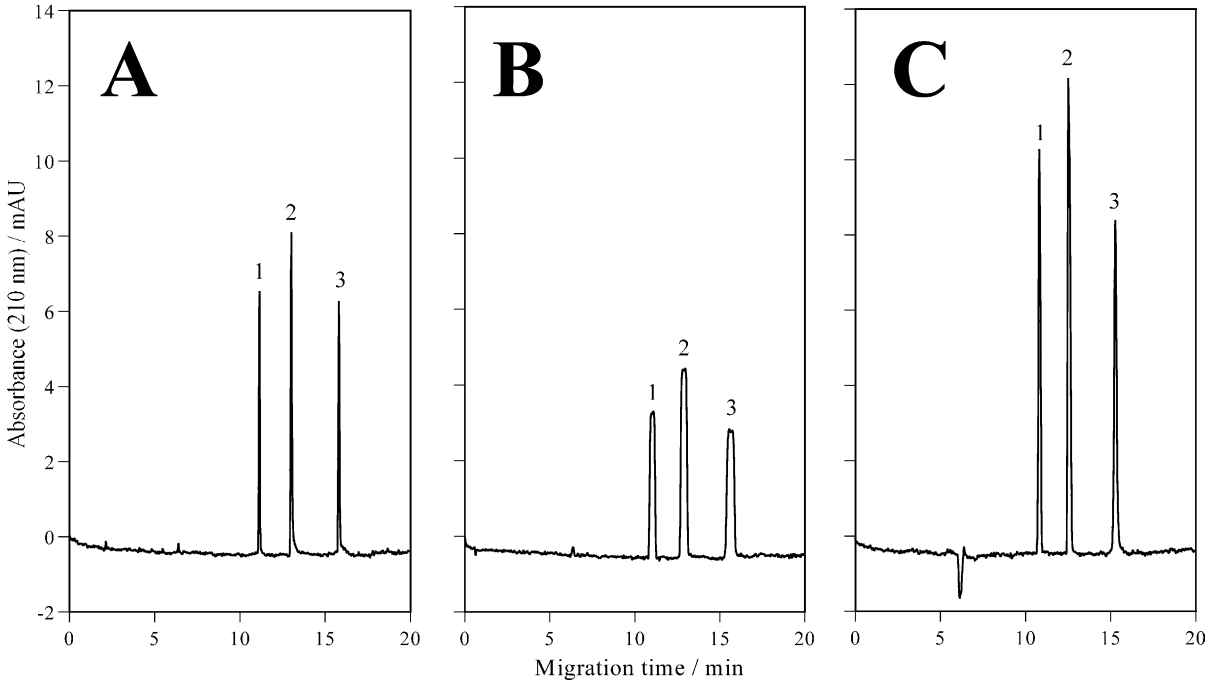

Fig. 4. Comparison of peak heights and shapes between different sample matrices. BGS, 50 mM phosphate (pH 7.0) containing 50 mM CTAC; sample matrix, (A, B) BGS, (C) water; injection time, (A) 1 s, (B, C) 30 s; concentration of samples, (A) nitrobenzene (peak 1, 157 ppm), resorcinol (peak 2, 122 ppm), 2-naphthol (peak 3, 58.5 ppm), (B, C) 10-fold dilution of samples in A; capillary, 61 cm (52.5 cm to detector) × 50 μm I.D.; detection, 210 nm; applied voltage, −20 kV; temperature, 25 °C.

NSAs were in the range from 0.47 to 0.96 ppb ($S/N = 3$) using a UV detector and without any preconcentration procedure. These LODs are almost the same level as that given in a previous report, where laser-induced fluorescence detection was used in combination with solid-phase extraction for the environmental analysis of NSAs in river water samples. The resolution was almost unaffected by sweeping even with sample plug lengths close to the effective length of the capillary. Sweeping analysis of *s*-triazine herbicides in MEKC using a cationic surfactant was evaluated by Lin et al. [30].

4.2.3. Nonionic micelles

Nonionic surfactants themselves do not possess electrophoretic mobility, but they have the distinct advantage of not contributing appreciably to Joule heating, hence, they may be used at high concentrations. They can have a great influence on the separation of charged analytes and can also be employed as PSs in MEKC in combination with ionic surfactants. Nonionic surfactants such as Brij 35 and Tween 20 were effectively used as PSs for on-line concentration techniques.

Three phenols (4-ethylphenol, 4-chlorophenol, and 3-methylphenol) were successfully separated and concentrated by NSM and sweeping MEKC using nonionic Brij 35 micelle [33]. Under the optimized condition, about 40- and 100-fold enhancements in detection sensitivity were obtained in terms of peak heights by NSM and sweeping MEKC, respectively.

4.2.4. Microemulsion

Oil-in-water (o/w) microemulsions have been shown to be good PS for EKC [64–66]. Microemulsions (o/w) are prepared by mixing oil, water,

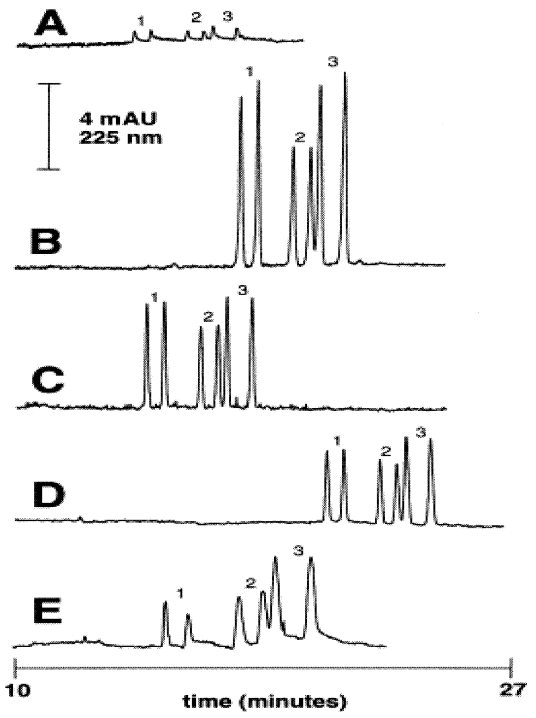

Fig. 5. Sample stacking and sweeping of phenoxy acid herbicides in CDEKC. BGS, 20 mM hepta-6-sulfato-β-CD in 15 mM phosphoric acid (pH 1.9). Sample S: phenoxy acid herbicides in water (A, B), in 1/40 dilution of the BGS (C, D), in phosphoric acid which has the same conductivity as the BGS (E). Peaks, fenoprop (1), mecoprop (2), dichlorprop (3). Concentration of analytes in A and E, ~50 ppm; concentration of analytes in B, C, and D, 1/10 diluted compared to A. Injection: 0.64 mm (A), 7.04 cm (B, SRMP), 0.96 cm of water followed by electrokinetic injection at −11 kV until 60% of the original current was reached (C, FESI–RMP), 2.88 cm water followed by 2.88 cm S (D, SRW), 0.45 cm (E, sweeping). Pressure, 50 mbar; applied potential, −11 kV.

a surfactant and a cosurfactant such as a medium alkyl-chain alcohol. They have characteristic properties as solvent, such as thermodynamic stability and high solubilization power. The structure of the o/w microemulsion is similar to that of the micelle, except that the microemulsion has a core of a minute droplet of an oil. The surfactant and the cosurfactant are located on the surface of the oil droplet to stabilize it. The separation basis of microemulsion EKC (MEEKC) is similar to that involved in MEKC where the surfactant monomers aggregate to form micelles. Sample stacking and sweeping of test steroids by MEEKC is shown in Fig. 9 [24].

5. Selective exhaustive injection sweeping

5.1. Cation selective exhaustive injection sweeping

Cation selective exhaustive injection–sweeping (CSEI–sweep) is a combination of two on-line preconcentration techniques, sample stacking with electrokinetic injection and sweeping, that can provide more than 100 000-fold increases in detection sensitivity [40,41]. In order to perform sample stacking with electrokinetic injection effectively, the sample has to be prepared in a low conductivity matrix.

The CSEI–sweep–MEKC model is illustrated in Fig. 10. The column is conditioned with a nonmicellar BGS. In step A, a zone of a high-conductivity buffer devoid of micelles (HCB) followed by a short water plug is introduced hydrodynamically. Here, the HCB zone provides a trap in which the analytes are stacked prior to being swept and separated in the micellar BGS, but does not affect the focusing effect of the sweeping step [40]. The water plug provides a high electric field at the tip of the capillary in sample stacking by electrokinetic injection, which will eventually improve the sample stacking procedure. In step B, the cation sample prepared in a low-conductivity solution is injected electrokinetically at positive polarity. The sample cations enter the capillary through the water plug with high velocities. Once the sample ions reach the interface between the water and HCB zones, they will slow down and focus at this interface. The continued electrokinetic injection builds up a long concentrated sample zone, which is too long to give high resolution unless it is refocused. It should be noted that the direction of the suppressed EOF is toward the anode but that of the cationic analyte migration is toward the cathode. In step C, once the separation voltage is applied at negative polarity with the micellar BGS in the inlet vial, anionic micelles enter the capillary and sweep the analytes. The stacked anions are completely swept by the

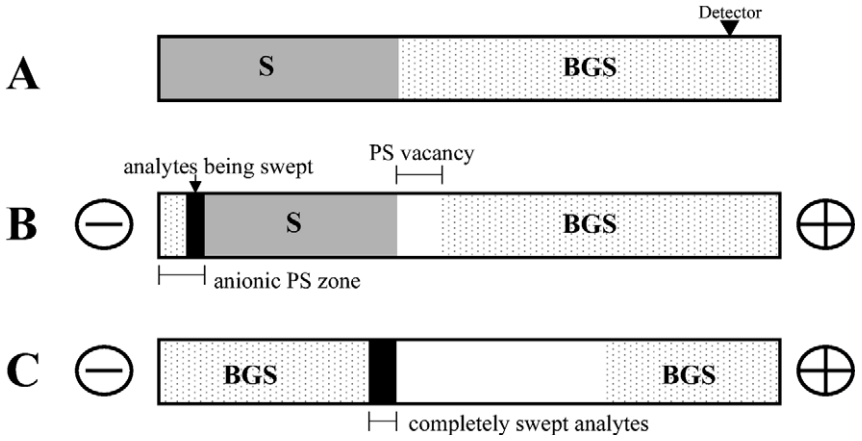

Fig. 6. Schematic diagram of the principle of sweeping under acidic condition. See text.

micelle and are separated by MEKC in the reverse migration mode.

Fig. 11 shows the electropherograms of tap water analysis by CSEI–sweep–MEKC [42]. The results of this study suggested that CSEI–sweep–MEKC can be used for the quaternary ammonium herbicides in drinking water.

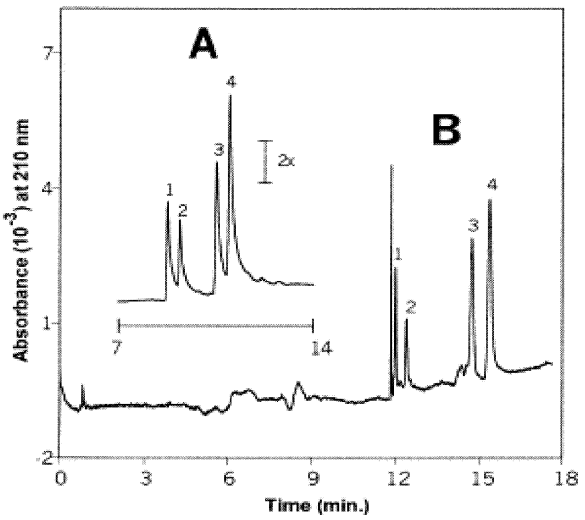

Fig. 7. Optimized electropherogram of selected biologically active compounds with sweeping and comparison with usual injection. Conditions: BGS, 100 mM SDS in 100 mM phosphoric acid/20% acetonitrile/2% methanol; S, trimipramine (1), nicardipine (2), noscapine (3), laudanosine (4) in phosphoric acid having a conductivity similar to the BGS; concentration of analytes, 190–265 ppm (A), 19–26.5 ppb (B); length of injected S, 0.064 cm (A), 42 cm (B); capillary, 50 μm (I.D.), 64.5 cm (total), 56 cm (effective); applied voltage, −23 kV.

5.2. Anion selective exhaustive injection sweeping

The principle of anion selective exhaustive injection (ASEI)–sweep–MEKC [31] technique is basically the same as CSEI–sweep–MEKC, but the procedure is slightly modified. First of all, to suppress the EOF, a polyacrylamide (PAA)-coated capillary is employed. In CSEI–sweep–MEKC, EOF is suppressed significantly under acidic conditions with anionic SDS micelles. However, the EOF is not suppressed even under acidic conditions in the presence of cationic micelles due to the strong adsorption of the cationic surfactant molecules on the surface of the fused silica capillary. In our previous report [27], EOF was successfully suppressed even under the neutral pH, when a PAA-coated capillary was employed. Therefore, a PAA-coated capillary is employed in the presence of cationic surfactant for ASEI–sweep–MEKC. In the next step, the anion sample prepared in a low-conductivity solution is injected electrokinetically at *negative polarity* and the separation voltage is applied at *positive polarity* with the micellar BGS in the inlet vial. The polarity for injection and separation is reversed compared to a CSEI–sweep–MEKC.

Some aromatic carboxylic acids, dansyl amino acids, and naphthalenedisulfonic acids were con-

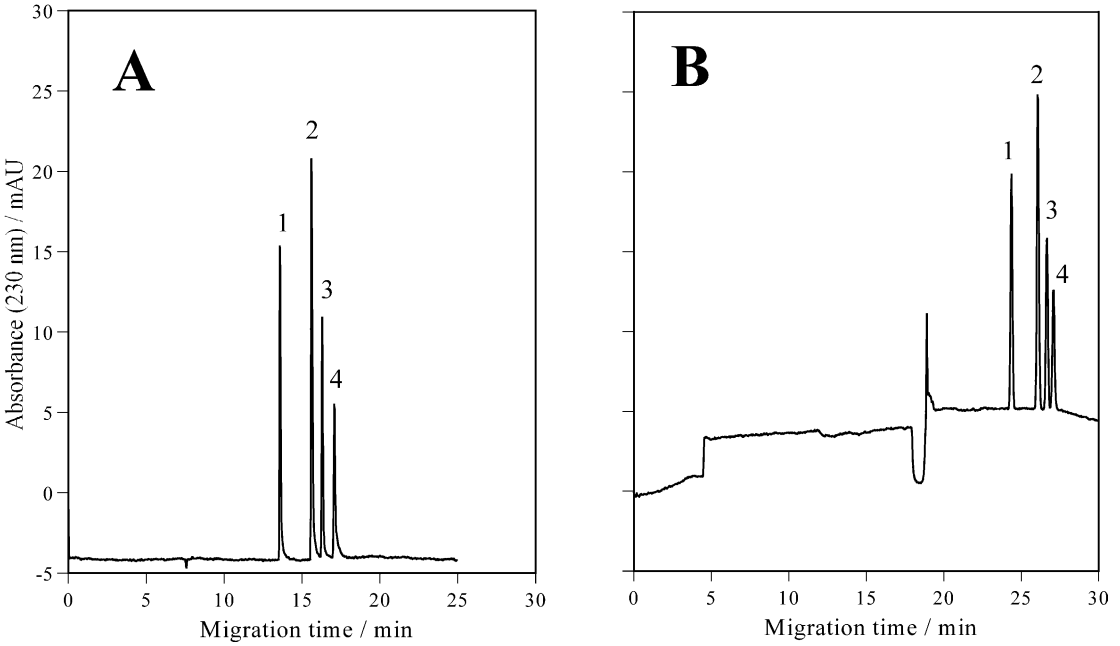

Fig. 8. Sweeping MEKC analysis of NSAs. BGS, 100 mM Tris–HCl (pH 7.0) containing 50 mM TTAB and 20% acetonitrile; sample solution, samples in Tris–HCl buffer (pH 7.0) having conductivity equal to that of the BGS (8.30 mS/cm); injected length, (A) 0.57 mm, (B) 45.6 cm; concentration of samples, (A) 2,6-NDSA (peak 1, 106 ppm), 1,5-NDSA (peak 2, 328 ppm), 2,7-NDSA (peak 3, 91 ppm), 1-NSA (peak 4, 112 ppm), (B) 1000-fold dilution of samples in A; detection, 230 nm; applied voltage, −15 kV; capillary, 50 μm I.D., 61 cm total (52.5 cm to detector); temperature, 25 °C.

centrated by ASEI–sweep–MEKC. Under the optimized conditions, about 1000–6000-fold increases in detection sensitivity were obtained by this technique [31].

6. Hyphenation technique with on-line preconcentration technique

6.1. Mass spectrometry

The use of mass spectrometry (MS) as a detection method in CE as well as EKC offers several advantages over UV detection method. Analytes having no strong UV absorption are detected with high sensitivity by MS detection. Furthermore, MS provides important information not only about molecular mass but also about structure of the analytes. The most important issue in CE-MS may be the development of interfaces between CE and MS. MS has been successfully coupled to CE with various interfaces [67–71]. An electrospray ionization (ESI) interface is widely employed in CE-MS systems [67,68]. However, the major drawback in EKC–MS with an ESI interface is that the introduction of a nonvolatile PS into the interface deteriorates ionization efficiency and contaminates the interface. To solve this problem, the use of partial filling technique has been reported [72,73]. Molina et al. [68] applied high-salt stacking technique to EKC–ESI-MS for the analysis of *N*-methylcarbamate pesticides. The optimum conductivity ratio between BGS and sample solution was found to be 2.7 and LODs were in the range of 0.04–2.0 ppm.

Ozaki and Terabe [70] have evaluated the feasibility of the atmospheric pressure chemical ionization (APCI) interface for EKC–MS system using SDS and several cholates as PSs, where the EKC–MS separation was successfully performed for some drug components. For highly sensitive analysis of environmental pollutants, Isoo et al.

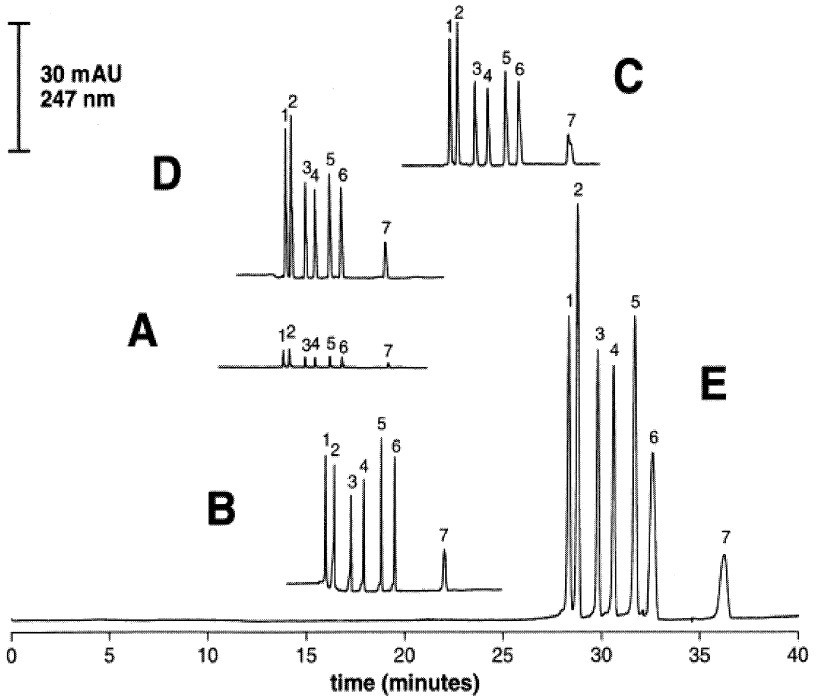

Fig. 9. Sample stacking and sweeping of test steroids in MEEKC. BGS: 100 mM SDS, 41 mM *n*-heptane, 700 mM 1-butanol in 50 mM phosphate buffer (pH 1.9). Steroids in sample (S): progesterone (1), testosterone (2), fluocinolone acetonide (3), betamethasone (4), hydrocortisone (5), cortisone (6), triamcinolone (7). Sample concentrations: ~90 ppm in the BGS (A), ~9 ppm in water (B), ~9 ppm in 1/10 dilution of the BGS (C, D), ~9 ppm in phosphoric acid solution which has the same conductivity as the BGS (E). Injection: 1-s or 0.64 mm (A, conventional injection), 7.04 cm (B, SRMP), 5.44 cm water and 6.4 cm S (C, SRW); 5.76 cm of water followed by electrokinetic injection at −18 kV until 80% of the original current is reached (D, FESI–RMP); 12.35 cm (E, sweeping). Separation conditions: applied voltage, −18 kV; injection pressure, 50 mbar (A, B, C, D), 1 bar (E); time scale of all electropherograms is the same.

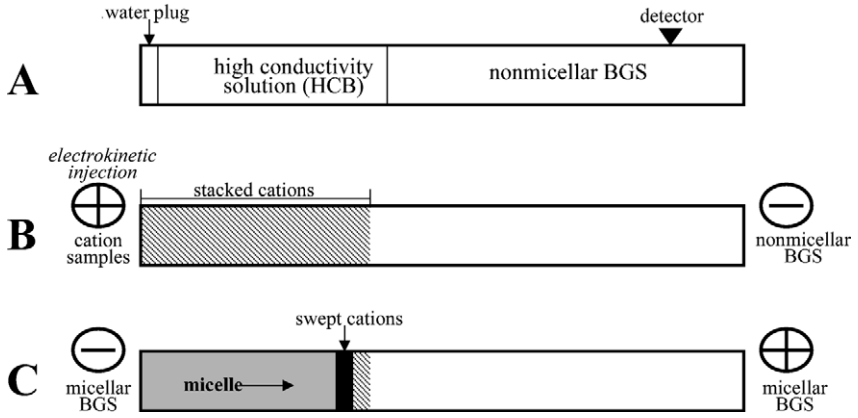

Fig. 10. Schematic diagrams of the CSEI-sweep-MEKC model. See text.

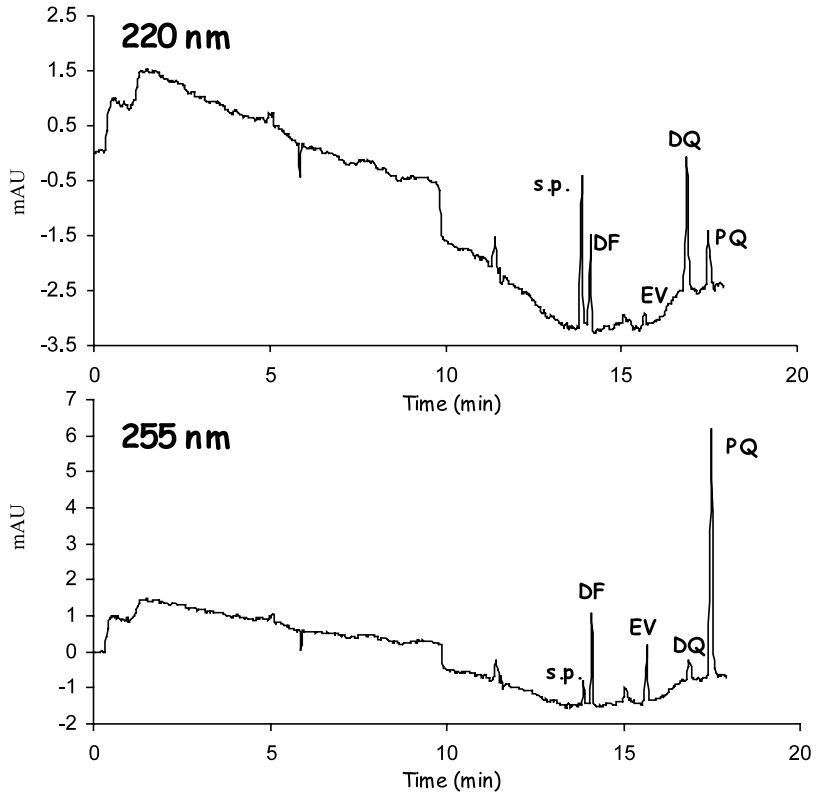

Fig. 11. Electropherogram of tap water analyzed by CSEI-Sweeping-MEKC. Nonmicellar BGS, 100 mM phosphate buffer (pH 2.5) containing 20% acetonitrile; micellar BGS, 80 mM SDS in 50 mM phosphate buffer (pH 2.5) containing 20% acetonitrile; HCB, 200 mM phosphate buffer (pH 2.5); conditioning solution before injection, nonmicellar BGS; sample concentration, 10 μg l^{-1} praquat (PQ), diquat (DQ) and ethylviologen (EV), 50 μg l^{-1} difenzoquat (DF); injection scheme: hydrodynamic injection of HCB for 200 s (5 kPa), hydrodynamic injection of water for 6 s (5 kPa), electrokinetic injection of Sample for 400 s (+22 kV); separation voltage, −22 kV with the micellar BGS at both ends of the capillary. s.p., system peak.

[44] reported an application study of sweeping to EKC hyphenated with MS using an APCI interface.

6.2. Microchip

Lately, microfabricated fluidicdevices (microchips) have received much attention and have been successfully demonstrated for CE [74–78]. In comparison with conventional CE, much shorter analysis times and smaller sample consumption were reported for these novel devices. However, as in conventional CE, detection sensitivity is also an important issue in microchip CE, although laser-induced fluorescence detectors are commonly used. To enhance the sensitivity of microchip CE devices, sample preconcentration can be performed prior to separation [76,77]. In microchip MEKC, on-line concentration of neutral analytes by electrokinetic stacking injections has been reported [78]. Recently, our research group successfully applied the sweeping technique to microchip MEKC [45]. In this paper, by changing the detection point along the separation channel, the profile of the concentration process and the diffusion process in sweeping were well evaluated. Based on these experiments, the width of the swept analyte zone was found to be extremely narrow and was equal to the size of focused laser spot (ca. 25 μm) in our equipment. The focused zone started to broaden immediately after the end of sweeping process due to thermal

diffusion. The dependence of zone broadening was accurately described by molecular diffusion. A sample electropherogram for the sweeping on a microchip MEKC analysis of rhodamine B, sulforhodamine B, and cresyl fast violet is shown in Fig. 12 [45].

7. Conclusion

Since the 1980s, MEKC has become the most popular technique for high-resolution separation of neutral and charged compounds. Relatively low detection sensitivity is one perceived limitation of MEKC, but various methods are available for solving this problem. Two on-line sample preconcentration techniques, sample stacking and sweeping, are shown to be attractive techniques for improvement of concentration sensitivity in MEKC. Various kinds of PSs have been used in MEKC for on-line sample preconcentration and such PSs should have different selectivities from each other.

In recent years, on-line coupling of sample stacking and sweeping MEKC with MS or microchip techniques attracted much attention. Other combination techniques with sample stacking and sweeping are also possible. One area of possibility is the combination of sweeping MEKC with dynamic pH-junction. Dynamic pH-junction [55,56] is a focusing method based on the difference in the analyte's ionization in the sample matrix and the BGS. For picomolar determination of some biologically important flavins, this technique was successfully demonstrated [79]. Another area of possibility is the on-line coupling of sample stacking or sweeping MEKC with inductively coupled plasma (ICP)-MS for the detection of trace elements. In reality, ICP-MS can provide quantitative elemental and isotopic information for most element. ICP-MS has been combined with CE and proves to be a useful detector [80–82].

In conclusion, several modes of on-line sample preconcentration in MEKC are now available. Moreover, the understanding of the on-line sample preconcentration mechanism will provide effective application of the techniques to the pharmaceutical, biomedical and environmental analysis, especially to diluted solutions. Therefore, such a capability will result in an extension of utility of MEKC as powerful analytical techniques.

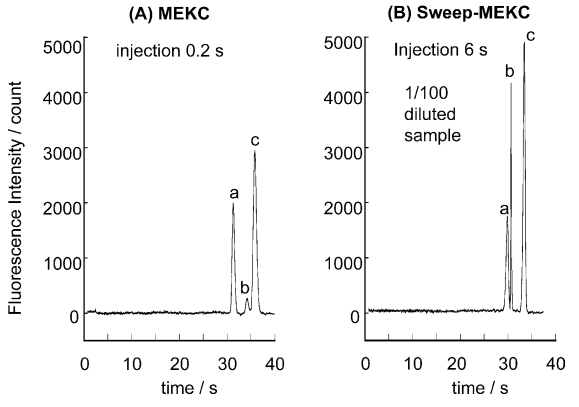

Fig. 12. On-line concentration and separation of the mixture of three fluorescent samples. (A) Normal MEKC, sample solution was the same as the running buffer. a, 1 μM sulforhodamine B; b, 10 μM cresyl fast violet; c, 1 μM rhodamine B. Injection time, 0.2 s. (B) Sweeping MEKC that sample zone contained no SDS micelles but was adjusted to the same conductivity as the running solution. a, 10 nM sulforhodamine B; b, 100 nM cresyl fast violet; c, 10 nM rhodamine B. Electric field, 370 V/cm; injection time, 6 s; distance of the detection point from the injection cross, 29.4 mm.

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